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(54) **Solid-phase enzyme-immunoassay method**

(57) Solid-phase enzyme-immunoassays, in which one of the components participating in the reaction is fixed to a solid phase, are improved by employing inthe system a buffer containing a polymer, such as polyethylene glycol or dextran, which accelerates the immunological antigen-antibody reaction.

GB 2 062 224 A

SPECIFICATION

Solid-phase enzyme-immunoassay method

The present invention relates to a method of performing enzyme-immunoassays, especially an improvement of the solid-phase enzyme-immunoassay (ELISA). In the method, one of the components participating in the reaction is fixed to a solid phase.

Solid-phase enzyme-immunoassays are nowadays widely used in hospitals and clinical laboratories for quantitative determination of different kinds of serum proteins, hormones and viral and bacterial antibodies in the IgG- and IgM-class, as well as drugs; in veterinary research institutes for the diagnosis of viral or bacterial infections in domestic animals and cattle; and in the field of agriculture, especially for the diagnosis of viral diseases in plants. The sensitivity and specificity of enzyme- and radioimmunological techniques are of the same order, but the reagents used in enzyme-immunoassays, unlike those used in radio-immunoassays, may be preserved for a long time and are not harmful to health (no radiation hazard). As, moreover, enzyme-immunological techniques do not require expensive equipment, specially trained staff, or special measures prescribed by law for the disposal of wastes, they have wide applicability, in addition to the fields of use mentioned above, in medical jurisprudence, the food industry, allergy research, and autoimmune serology.

The use of enzyme-immunoassays has been previously described, for instance in the following patents of Organon: US 3,879,262, US 3,791,932, US 3,850,752 and US 3,839,153. These assays are characterized by the use of antigens or antibodies fixed to a solid phase and the determination of their antibodies or antigens by using enzyme-labelled anti-immunoglobulin. In these methods, however, the slowness of the antigen-antibody reactions is a drawback, particularly in such cases where a quick diagnosis is needed to enable prompt therapeutic measures. Until now, the reaction rate has been accelerated by letting the reaction take place at +37°C in a heating cabinet.

We have now found that the sensitivity of solid phase enzyme-immunoassays can be improved by using as reagent a buffer containing a polymer which accelerates the immunological antigen-antibody reaction.

This means that by using the present invention the antigen-antibody reactions can be performed rapidly at room temperature without any reduction of sensitivity as compared to the methods used before. The invention extends the range of applicability of enzyme-immunoassays and makes them feasible even in primitive conditions.

For instance, a reaction carried out between enzyme-labelled anti-immunoglobulin and immunoglobulin G in a buffer containing an accelerating polymer according to the present invention proceeds at least five times faster than one carried out under similar conditions in a buffer not containing such a polymer. Polymers exert a promoting effect on the interaction between soluble antigens and antibodies apparently by steric exclusion of the immune complexes from the domain of the polymer.

As a polymer which accelerates the immunological antigen-antibody reaction, polyethylene glycol has proved extremely useful, but other polymers, e.g. dextran, polyvinylpyrrolidone (PVP), polyvinylalcohol (PVA), or some other polymer influencing immunological reactions, can be used as well.

Depending on the size and kind of protein to be determined it has been found preferable to use 0.01—20% (w/v) accelerating polymer, e.g. in 0.01M phosphate buffer at pH 7.2, containing 0.9% (w/v) NaCl and 0.1% (w/v) NaN_3.

The invention is illustrated by the following Examples:

EXAMPLE 1

Determination of Viral Antibodies by Using a Buffer Containing Polyethylene Glycol (PEG 6000)

1. The virus or its component is fixed to polystyrene (e.g. tubes, cuvettes or microtitre plates) by diluting it with a 0.01M phosphate buffer (pH 7.2), containing 0.9% (w/v) NaCl, in doing which the virus or its component, in a volume of 200 μl, is passively absorbed to the polystyrene. After 16—20 hours the excess antigen or its component is washed off by rinsing the tubes twice with distilled water.

2. The serum specimens are diluted 1:200 in 0.01M phosphate buffer (pH 7.2), containing 4% (w/v) polyethylene glycol (PEG 6000), 0.9% (w/v) NaCl, 0.1% (v/v) Tween 20 and 0.1% (w/v) NaN_3.

3. The diluted serum specimens (200 μl) are allowed to react with the antigen for 30 minutes at room temperature, after which the tubes are washed by rinsing twice with distilled water.

4. The conjugate (anti-human IgG labelled with alkaline phosphatase) is diluted 1:200 in 0.01M phosphate buffer (pH 7.2), containing 4% (w/v) polyethylene glycol (PEG 6000), 0.9% (w/v) NaCl, 0.1% (v/v) Tween 20 and 0.1% (w/v) NaN_3.

5. The conjugate (200 μl) is allowed to react for 1 hour at room temperature with the specific antibodies of the antigen in the sample, after which the tubes are rinsed with distilled water.

6. Disodium salt of alkaline phosphate is dissolved in diethanolamine $MgCl_2$ buffer (200 mg/100 ml buffer) and 200 μl of this solution is added into tubes and incubated 30 minutes at room temperature.

7. The enzyme reaction is stopped with 1M NaOH (200 μl) and the intensity of the colour formed is measured spectrophotometrically or examined visually (in a series of samples a known positive and negative reference sample treated as above is always included).

EXAMPLE 2

Determination of Viral Antibodies by Using a Buffer Containing Dextran (Dx 150)

1. The virus or its component is fixed to

polystyrene as in Example 1 paragraph 1.

2. The serum specimens are diluted 1:200 in 0.01M phosphate buffer (pH 7.2), containing 9% (w/v) dextran (Dx 150), 0.9% (w/v) NaCl, 0.1% (v/v) Tween 20 and 0.1% (w/v) NaN_3.

3. The diluted serum specimens are allowed to react as in Example 1 paragraph 3.

4. The conjugate (anti-human IgG labelled with alkaline phosphatase) is diluted 1:200 in 0.01M phosphate buffer pH 7.2, containing 9% (w/v) dextran (Dx 150), 0.9% (w/v) NaCl, 0.1% (v/v) Tween 20 and 0.1% (w/v) NaN_3.

5. The determination is continued as in Example 1 paragraphs 5—7.

EXAMPLE 3

Determination of C-Reactive Protein (CPR) by Using a Buffer Containing Polyethylene Glycol (PEG 6000)

1. Rabbit anti-human CRP is fixed to the surface of a plastic tube by diluting 1:8000 (200 μl) in 0.01M phosphate buffer pH 7.2, containing 0.9% (w/v) NaCl. The antiserum dilution is incubated at room temperature, at which the antibody is passively absorbed to the polystyrene. Incubation time is 16—20 hours at room temperature. Excessive antibody is washed off by rinsing the tubes twice with distilled water.

2. The serum samples are diluted 1:1000 in 0.01M phosphate buffer (pH 7.2), containing 3% (w/v) polyethylene glycol (PEG 6000), 0.9% (w/v) NaCl, 0.1% (v/v) Tween 20, and 0.1% (w/v) NaN_3.

3. The diluted serum samples (200 μl) are incubated with the antibody for 30 minutes at room temperature, after which the tubes are washed by rinsing twice with distilled water.

4. Swine anti-human CRP is diluted 1:1500 (200 μl) in 0.01M phosphate buffer (pH 7.2), containing 4% (w/v) polyethylene glycol (PEG 6000), 0.9% (w/v) NaCl, 0.1% (v/v) Tween 20 and 0.1% (w/v) NaN_3. After one hour's incubation the interaction between CRP and anti-CRP takes place at room temperature. The tubes are washed twice with distilled water as above.

5. Conjugate (alkaline-phosphatase-labelled Sheep anti-swine IgG) is diluted 1:200 in 0.01M phosphate buffer (pH 7.2), containing 4% (w/v) polyethylene glycol (PEG 6000), 0.9% (w/v) NaCl 0.1% (v/v) Tween 20 and 0.1% (w/v) NaN_3.

6. The conjugate (200 μl) is allowed to react with swine anti-human CRP for 1 hour at room temperature, after which the tubes are washed as above.

7. Disodium salt of alkaline phosphate is dissolved in diethanolamine $MgCl_2$ buffer (200 mg/100 ml buffer) and 200 μl of this solution is added to the tubes and incubated 30 minutes at room temperature.

8. The enzyme reaction is stopped with 1M NaOH (200 μl) and the intensity of the colour is measured spectrophotometrically or examined visually.

CLAIMS

1. A method of performing a solid-phase enzyme-immunoassay in which one of the components participating in the reaction is fixed to a solid phase, which comprises employing a buffer containing a polymer which accelerates the immunological antigen-antibody reaction.

2. A method according to claim 1, wherein the accelerating polymer is polyethylene glycol or dextran.

3. A method according to claim 1 or 2 wherein the buffer contains 0.01—20% (w/v) accelerating polymer.

4. A method according to claim 3 wherein the buffer is 0.01M phosphate buffer at pH 7.2, containing 0.9% (w/v) NaCl and 0.1% (w/v) NaN_3.

5. A method according to claim 1 carried out substantially as described in any of the foregoing Examples.

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